Antagonistic effects of atipamezole and yohimbine on medetomidine-induced diuresis in healthy dogs

Md. Hasanuzzaman Talukder, Yoshiaki Hikasa, Hajime Takahashi, Kanako Sato, Aya Matsuu

Abstract

This study aimed to investigate and compare the antagonistic effects of atipamezole and yohimbine on medetomidine-induced diuresis in healthy dogs. Five dogs were used repeatedly in each of 8 groups. One group was not medicated. Dogs in the other groups received 20 μg/kg of medetomidine intramuscularly and, 0.5 h later, saline (as the control injection), 50, 100, or 300 μg/kg of atipamezole, or 50, 100, or 300 μg/kg of yohimbine intramuscularly. Urine and blood samples were taken 11 times over 24 h for measurement of the following: urine volume, specific gravity, and creatinine concentration; urine and plasma osmolality; urine and plasma concentrations of electrolytes and arginine vasopressin (AVP); and the plasma concentration of atrial natriuretic peptide (ANP). Both atipamezole and yohimbine antagonized the diuretic effect of medetomidine, inhibiting medetomidine-induced decreases in urine specific gravity, osmolality, and concentrations of creatinine, sodium, potassium, chloride, and AVP and reversing both the medetomidine-induced increase in plasma concentrations of sodium, potassium, and chloride and the medetomidine-induced decrease in the plasma AVP concentration. Atipamezole significantly stimulated ANP release. The antidiuretic action of yohimbine was more potent than that of atipamezole but was not dose-dependent, in contrast to the action of atipamezole. The effects of these drugs may not be due only to actions mediated by α_2-adrenoceptors.

Résumé

L'objectif de la présente étude était d'examiner et de comparer les effets antagonistes de l'atipamezole et de la yohimbine sur la diurèse induite par la medetomidine chez des chiens en santé. Cinq chiens ont été utilisés de manière répétée dans chacun de 8 groupes. Un groupe n'était pas médicamenté. Les chiens dans les autres groupes ont reçu 20 μg/kg de médétomidine par voie intramusculaire et, 0,5 h plus tard, de la saline (injection témoin), 50, 100 ou 300 μg/kg d'atipamézole, ou 50, 100 ou 300 μg/kg de yohimbine par voie intramusculaire. De l'urine et des échantillons de sang ont été prélevés 11 fois sur une période de 24 h et les paramètres suivants ont été mesurés : le volume, la densité et la concentration de créatinine urinaire; l'osmolarité urinaire et plasmatique; les concentrations urinaire et plasmatique en électrolytes et d'arginine vasopressine (AVP); et la concentration plasmatique du peptide natriurétique atrial (ANP). L'atipamezole et la yohimbine avaient un effet antagoniste sur l'effet diurétique de la medetomidine en inhibant la diminution induite par la medetomidine de la densité urinaire, de l'osmolarité et des concentrations de créatinine, sodium, potassium, chlorure et AVP et en renversant l'augmentation des concentrations plasmatiques de sodium, potassium et chlorure et la diminution de la concentration plasmatique d'AVP causées par la medetomidine. L'atipamezole a stimulé de manière significative la relâche d'ANP. L'effet antidiurétique de la yohimbine était plus marqué que celui de l'atipamezole mais n'était pas dose dépendant contrairement à l'action de l'atipamezole. Les effets de ces médicaments ne sont peut-être pas uniquement associés à des mécanismes à médiation par des adrénorécepteurs α_2.

(Traduit par Docteur Serge Messier)

Introduction

The α_2-adrenoceptors are the transmembrane G-protein-coupled receptors that act pre- or post- and extrasynaptically in different tissues (1). Pharmacologic subtypes (α_{2A}, α_{2B}, α_{2C}, and α_{2D}) of the α_2-adrenergic receptors have been identified on the basis of ligand affinity (2). Some ligands have an imidazole or imidazoline ring that enables them to bind to nonadrenergic, imidazole-preferring receptors as well as to α_2-adrenoceptors (3). Medetomidine is the prototype of the novel selective α_2-adrenoceptor agonists with imidazoline-receptor affinity (4). The α_2/α_1 receptor-binding selectivity ratio of medetomidine is 1620/1, compared with 160/1, 260/1, and 220/1 for xylazine, detomidine, and clonidine, respectively (5). Medetomidine is also more lipophilic than xylazine, detomidine, and clonidine (6). It has been widely used for sedation, analgesia, muscle relaxation, immobilization, and reduction of peristalsis during gastrointestinal surgery and endoscopy in veterinary practice (7,8).

The α_2-adrenoceptor antagonists atipamezole and yohimbine have been shown to reverse a variety of clinicophysiological effects produced by α_2-adrenoceptor agonists (1,4,5,7,9–11). The α_2/α_1 selectivity ratios of atipamezole and yohimbine are 8526/1 and 40/1, respectively (5). Atipamezole is a potent and highly specific antagonist of

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centrally and peripherally located α_2-adrenoceptors (10). Whereas the affinities of atipamezole and yohimbine are similar at the α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors, atipamezole has approximately 100 times more affinity than yohimbine at the α_{2D}-adrenoceptors (5). Yohimbine affects serotonergic, cholinergic, dopaminergic, and γ-aminobutyric acid receptor-related mechanisms (11); atipamezole lacks these receptor activities (10). Furthermore, atipamezole has a structure similar to that of imidazoline, whereas yohimbine has no imidazoline-receptor affinity (1–5,10,12). These differences between atipamezole and yohimbine may influence their antagonistic effects on medetomidine-induced actions.

Medetomidine is known to induce diuresis in several species, including dogs (13–15). Recently we found that the changes induced by this agent in urine specific gravity, pH, creatinine concentration, and osmolality, as well as the changes in concentrations of sodium, potassium, and chloride in both urine and plasma, are dose-dependent in healthy dogs (16). In that study we also demonstrated that medetomidine decreases in a dose-dependent manner the urinary excretion of arginine vasopressin (AVP) and suppresses the plasma concentration of AVP; this drug also increases the plasma concentration of atrial natriuretic peptide (ANP) in a dose-dependent manner at the early phase after administration. The results of previous studies and our recent study have suggested that the decrease in the plasma concentration of AVP plays a partial role in the profound diuretic effect of medetomidine through its action on α2-adrenoceptors (13,14,16–19). Medetomidine has been also reported to markedly induce ANP release in normotensive rats (20). Primarily of cardiac origin, ANP is a potent vasodilator, diuretic, and natriuretic hormone (21). In our recent article we claimed that ANP may partially influence the medetomidine-induced diuresis in dogs, because it reportedly exerts a diuretic and natriuretic action on the proximal tubules and inner medullary duct cells of the kidney in rats (21–24) and mice (25). The sedative and analgesic actions of medetomidine are accompanied by initial hypertension and then prolonged hypotension, respiratory acidosis, and hypoxemia in several species of animals (7,9). It is unknown whether these actions may influence the diuretic effect induced by medetomidine.

The regulation of water excretion has implications for a number of clinical situations. However, to the best of our knowledge, there are no published data on the antagonistic effects of atipamezole and yohimbine on medetomidine-induced diuresis in dogs. In addition, there is no report that either atipamezole or yohimbine reverses the inhibition of AVP and the release of ANP induced by medetomidine in dogs. This study aimed to investigate and compare the antagonistic effects of 3 different doses of either atipamezole or yohimbine on the diuresis induced by medetomidine in healthy dogs. The variables examined were urine volume, specific gravity, and creatinine concentration, osmolality and concentrations of electrolytes and AVP in both urine and plasma, and concentrations of ANP in plasma.

Materials and methods

Animals

Five healthy adult male dogs, 2 beagles and 3 dogs of mixed breed, with a mean age of 5.8 [standard deviation (*s*) = 2.7] y and a mean weight of 11.2 (*s* = 1.8) kg were used. All the dogs were raised at a laboratory providing animal management facilities and fed a standard commercial dry canine food. Routine hematologic examination before the study showed all values to be within the normal physiological ranges (26). The experimental protocol was approved by the Animal Research Committee of Tottori University, Tottori, Japan.

Experimental protocol

The 5 dogs were assigned to each of 8 treatment groups in a randomized design, with 1 wk between treatments for each dog. In 1 group each dog was given an intramuscular (IM) injection of 2.0 mL of physiological saline solution as the nonmedicated control treatment. In the other groups each dog received an IM injection of 20 μg/kg of medetomidine hydrochloride (0.1% solution; Domitor, Meiji Seika, Tokyo, Japan) and then, 0.5 h later, an IM injection of one of the following: 0.5 mL of physiological saline solution; 50, 100, or 300 μg/kg of atipamezole hydrochloride (0.5% solution; Antisedan, Meiji Seika); or 50, 100, or 300 μg/kg of yohimbine hydrochloride (Sigma Chemical, St. Louis, Missouri, USA) dissolved in distilled water for a concentration of 0.5 mg/mL. The groups will be referred to as Saline, MED, MED-ATI 50, MED-ATI 100, MED-ATI 300, MED-YOH 50, MED-YOH 100, and MED-YOH 300. Because IM injection has often been used for α_2-adrenoceptor agonists (27), this route was chosen for our study. The quadriceps muscle was used for the injection.

The dogs were fasted for 12 h before the experiment. After sample collection at 8 h, feeding was done once, and then the dogs were again fasted for 12 h before the 24-h sample was collected the next day. The experiment was performed in a room with the air temperature at 25°C.

Sample collection

A 6- or 8-Fr silicon balloon catheter (All Silicon Foley Catheter; Create Medic Company, Yokohama, Japan) was inserted 1 h before treatment to empty the bladder and for subsequent urine sampling. The catheter was withdrawn after sampling at 8 h. The next day, at 22 h, the catheter was again inserted and the bladder emptied; a urine sample was collected at 24 h. Urine and blood samples were taken at the following 11 times: before injection of the agent (0 h) and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h after the injection of medetomidine. The urine samples were centrifuged at 2000 × *g* for 5 min, and then the supernatant was collected and stored at -40°C until analyzed. Blood samples (5.5 mL) were collected from the jugular vein by means of a 21-gauge needle with a 6-mL disposable syringe; a 4.0-mL aliquot was mixed with ethylene diamine tetraacetic acid and aprotinin (Trasylol; Bayer, Leverksen, Germany) for AVP and ANP measurements, and the remaining 1.5 mL was mixed with heparin for osmolality measurement. The blood samples were immediately centrifuged at 2000 × *g* at 4°C for 15 min, and then the plasma was separated and kept at -40°C until analyzed.

Analytical methods

Urine volume was measured at each time point in a measuring cylinder after collection from the urine bag. Specific gravity and pH were measured by a refractor photometer (Erma, Tokyo, Japan) and a pH meter (F-52; Horiba STEC, Santa Clara, California, USA),

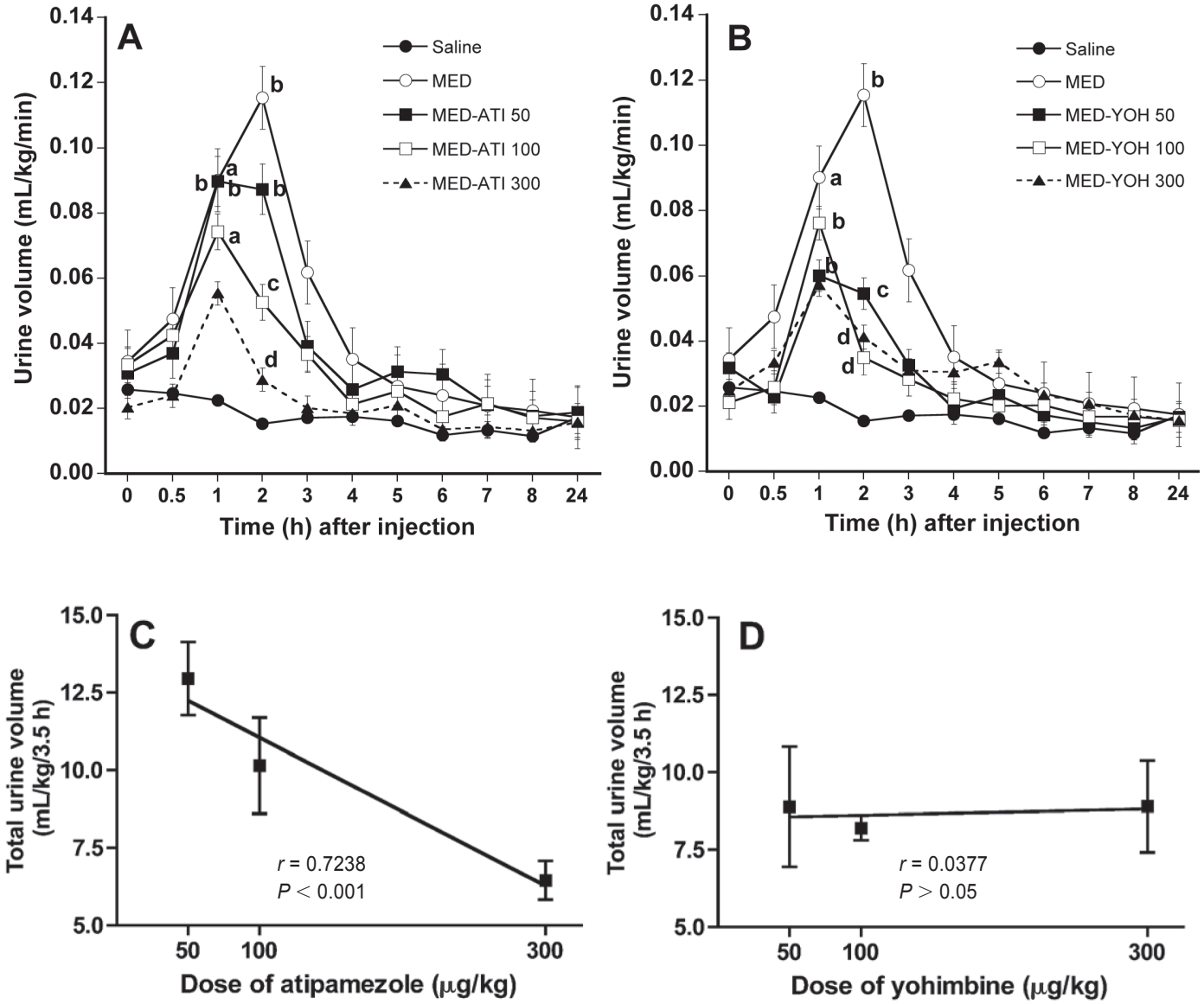

Figure 1. Urine volume in 5 dogs before (0) and after intramuscular injection of physiological saline (Saline) or medetomidine followed, 0.5 h later, by saline again (MED) or by atipamezole (ATI) or yohimbine (YOH), the last 2 drugs in doses of 50, 100, or 300 μg/kg. A, B: Each point and vertical bar represent the mean and standard error (n = 5) of the rate of diuresis at various time points. The letters indicate a significant difference from the baseline (0) value (a — $P < 0.05$; b — $P < 0.01$) or from the value for the MED group (c — $P < 0.05$; d — $P < 0.01$). The points, bars, and letters have the same meanings in Figures 2 through 6. C, D: Simple linear regression of total urine volume in the period 0.5 to 4 h after the injection of medetomidine.

respectively. Urine creatinine concentration was measured with an assay kit (Wako Pure Chemical Industries, Osaka, Japan) by the Jaffe method, with the use of a spectrophotometer. In both urine and plasma, osmolality and electrolyte concentrations were measured by means of a vapor pressure osmometer (Vapro; Wescor, Logan, Utah, USA) and an Na–K–Cl ion-concentrations autoanalyzer (Dri-Chem 800V; Fuji Film Company, Tokyo, Japan), respectively.

Plasma AVP was extracted by solid-phase column extraction with the use of Sep-Pak cartridges (Waters, Milford, Massachusetts, USA). Urine AVP extraction was not needed. The urine and plasma AVP concentrations were measured by double-antibody radioimmunoassay (RIA) with a commercially available kit (Mitsubishi Chemical, Tokyo, Japan). The intra-assay coefficients of variation (CVs) were 10% and the limits of detection and quantification, 0.063 to 8.0 pg/mL, respectively. Plasma ANP was also assayed with a double-antibody RIA kit (HANP kit; Eiken Chemical, Tokyo, Japan). The intra-assay CV was 15%, and the detection and quantification limits were 10 and 1280 pg/mL, respectively.

Data evaluation

All data obtained were analyzed together with Prism statistical software (version 4; GraphPad Software, San Diego, California, USA). One-way analysis of variance for repeated measures was used to examine the time effect within each group and the group effect at each time point. When a significant difference was found, the Tukey test was used to compare the means. The area under the curve (AUC) was calculated for each biochemical variable. The AUC was measured by calculating the sum of the trapezoids formed by the data

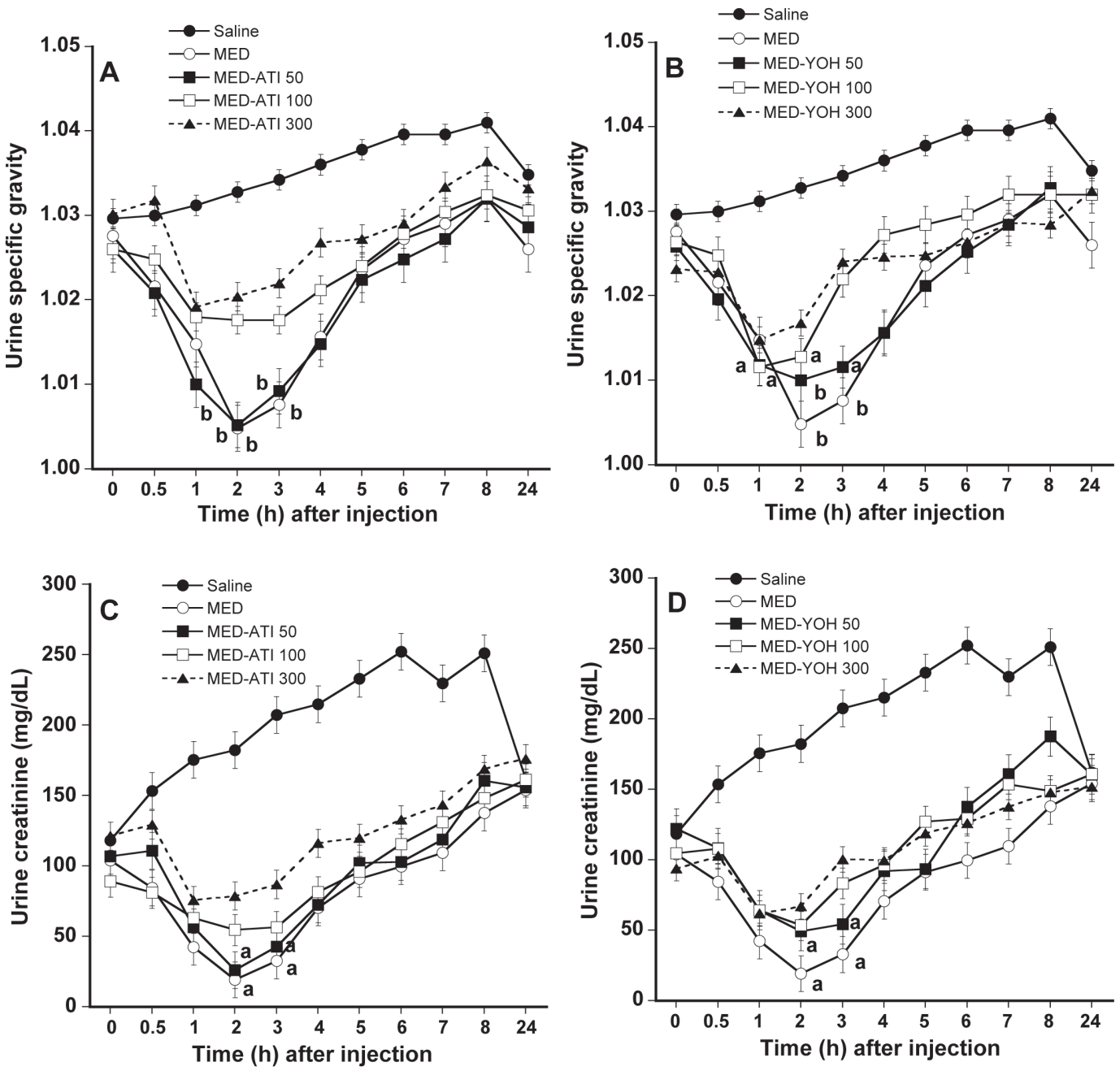

Figure 2. Urine specific gravity (A, B) and creatinine concentration (C, D) before and after injection of saline or medetomidine.

points. The AUC data were plotted against the doses of atipamezole or yohimbine, and simple linear regression analysis was applied. When a significant difference was found, the effect of the drug on the level of the examined biochemical variable was claimed to be dose-dependent. Mean values are presented with standard error ($s_{\bar{x}}$) in parenthesis. The level of significance in all tests was set at $P < 0.05$.

Results

For all the variables, there were no significant differences between the groups at baseline (0 h). No significant changes in urine volume and other biochemical and hormonal variables were observed in the Saline group. Medetomidine significantly increased urine production at 1 and 2 h, and this effect persisted for up to approximately 4 h after injection (Figures 1A and 1B). The peak means for urine volume demonstrated a significant inhibition of medetomidine-induced diuresis in all the medicated groups except MED-ATI 50. Moreover, the linear regression of the total urine volume from 0.5 to 4 h was highly significant ($P < 0.001$) in the MED-ATI groups but not in the MED-YOH groups, indicating that atipamezole but not yohimbine inhibited medetomidine-induced diuresis in a dose-dependent manner at the tested doses (Figures 1C and 1D). Similar results

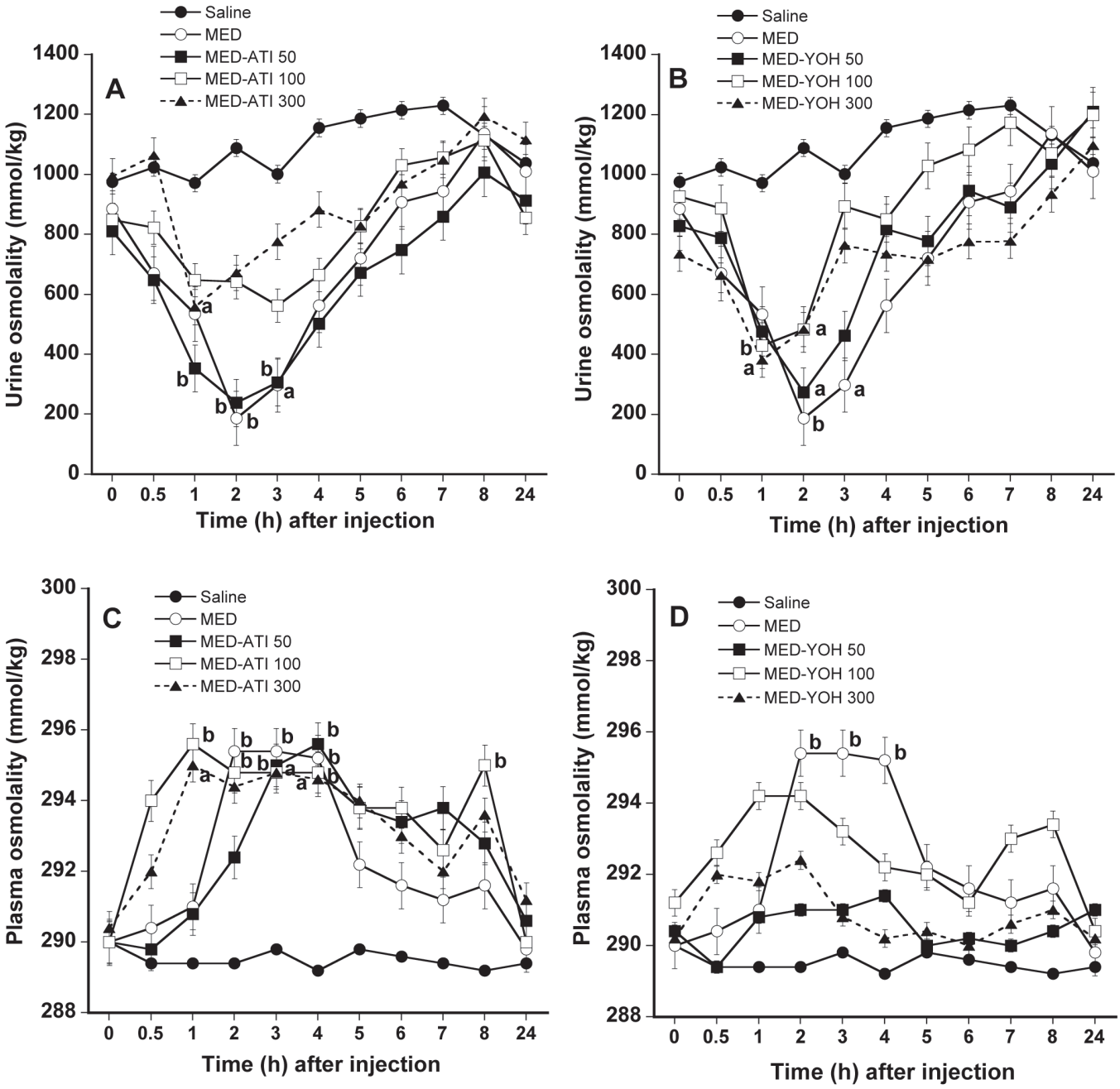

Figure 3. Osmolality of urine (A, B) and plasma (C, D) before and after injection of saline or medetomidine.

were observed with linear regression of the total urine volume from 0.5 to 2 h, 0.5 to 3 h, 0.5 to 6 h, and 0.5 to 8 h.

The urine specific gravity decreased significantly from 0.5 to 4 h after the injection of medetomidine in the MED, MED-ATI 50, and MED-YOH 50 groups but not the other groups (Figures 2A and 2B). The decreases in urine specific gravity corresponded to the decreases in urine volume.

The urine creatinine concentration increased gradually over the first 8 h after the injection of saline alone, whereas it decreased significantly in the MED, MED-ATI 50, and MED-YOH 50 groups over the first 2 to 3 h (Figures 2C and 2D). The lowest mean concentration was at 2 h in the MED-ATI 50 and MED-YOH 50 groups. Both atipamezole and yohimbine reduced in a dose-dependent manner the decrease in urine creatinine concentration induced by medetomidine. The urine osmolality in the MED-ATI and MED-YOH groups decreased significantly and similarly over the first 1 to 3 h after the injection of medetomidine (Figures 3A and 3B). Higher doses of both atipamezole and yohimbine reduced the decrease. On the other hand, the plasma osmolality in the MED group increased significantly over the first 2 to 4 h after the injection of medetomidine (Figures 3C and 3D), and all doses of yohimbine prevented the increase.

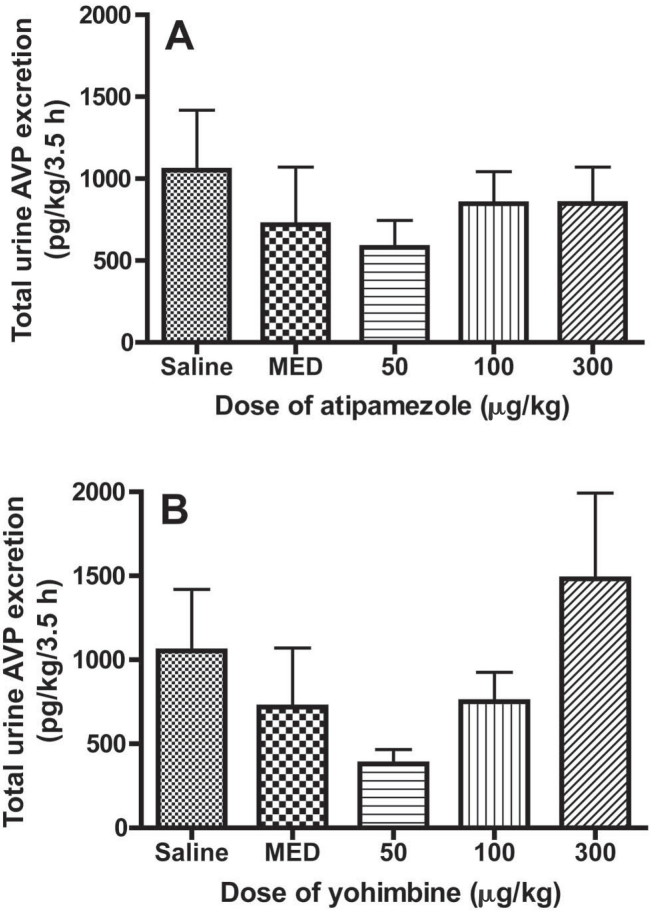

Figure 4. Total urine excretion of arginine vasopressin (AVP) between 0.5 and 4 h after the injection of saline or medetomidine. On the x axis, "50", "100", and "300" indicate the dose, in micrograms per kilogram, of the 2nd drug injected: atipamezole (A) or yohimbine (B); the same explanation applies in Figures 5 and 6.

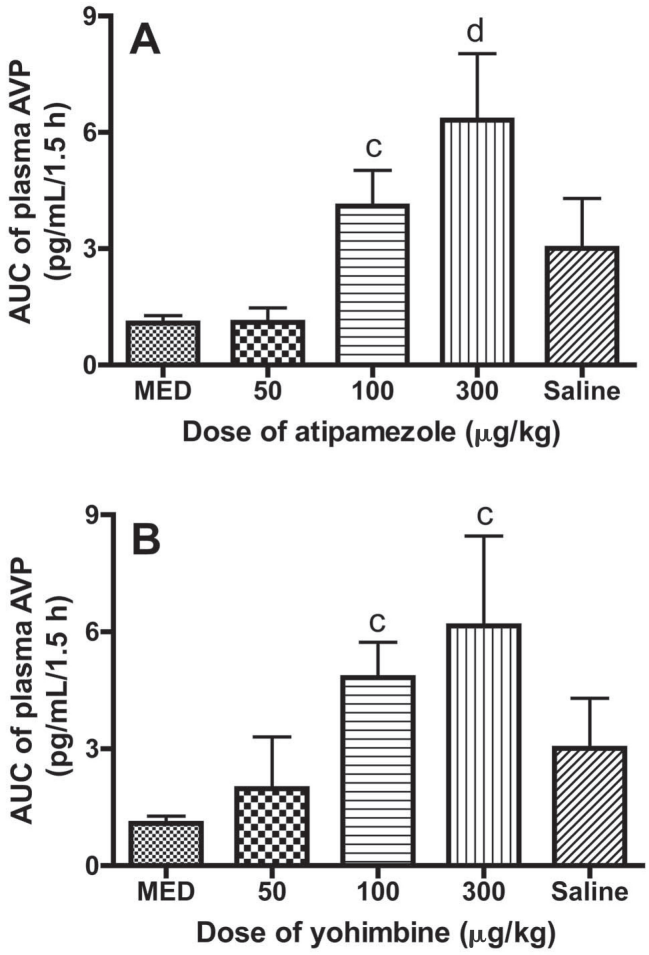

Figure 5. The area under the curve (AUC) for the plasma AVP concentration between 0.5 and 2 h after the injection of saline or medetomidine.

The total amount of urine AVP excreted between 0.5 and 4 h after the injection of saline or medetomidine was lower in the MED group than in the Saline group (Figures 4A and 4B). There were no significant differences in the total amount of urine AVP excreted between the MED and MED-ATI groups (Figure 4A). However, yohimbine reversed the medetomidine-induced decrease in AVP excretion in a dose-dependent manner (Figure 4B). The linear regression of the total urine AVP excretion between 0.5 and 4 h was significant in the MED-YOH groups ($r = 0.5809$; $P < 0.05$) but not in the MED-ATI groups ($r = 0.2026$; $P > 0.05$). Similar results were obtained with linear regression of the total urine AVP excretion from 0.5 to 2 h, 0.5 to 3 h, and 0.5 to 8 h.

The AUC for the plasma AVP concentration from 0.5 to 2 h after the injection of saline or medetomidine was significantly higher in the MED-ATI 100 and 300 groups and the MED-YOH 100 and 300 groups than in the MED group (Figures 5A and 5B). Similar results were obtained for the AUC data obtained from 0.5 to 3 h and from 0.5 to 4 h. The linear regression of the AUC data from 0.5 to 2 h was highly significant in the MED-ATI groups ($r = 0.6249$; $P < 0.01$) but not in the MED-YOH groups ($r = 0.4054$; $P > 0.05$) in all the groups. These results show that both atipamezole and yohimbine reversed the medetomidine-induced decrease in plasma AVP concentration. Similar results were found with the linear regression of the AUC data from 0.5 to 3 h and from 0.5 to 4 h.

The AUC for the plasma ANP concentration from 0.5 to 4 h after the injection of saline or medetomidine was significantly increased in the MED-ATI 300 group compared with the MED group, whereas no dose of yohimbine significantly altered ANP release. The linear regression of the AUC data showed that atipamezole increased ANP release in a dose-dependent manner, whereas yohimbine did not (Figures 6A and 6B). Similar results were obtained with the linear regression of AUC data for plasma ANP from 0.5 to 2 h and from 0.5 to 3 h.

In the MED group, the mean urine concentrations of sodium, potassium, and chloride were lower than the baseline values over the first 0.5 to 4 h after the injection of saline or medetomidine and then increased beyond the baseline values in the period of 5 to 8 h. Higher doses of atipamezole or yohimbine prevented the decreases in the urine concentrations of sodium, potassium, and chloride induced by medetomidine (Table I) but did not significantly change the urine concentrations of sodium, potassium, and chloride at 24 h. The total amounts of sodium, potassium, and chloride excreted in the urine between 1 and 3 h after the injection of saline or medetomidine were not significantly different in the MED-ATI or MED-YOH groups compared with the MED and Saline groups.

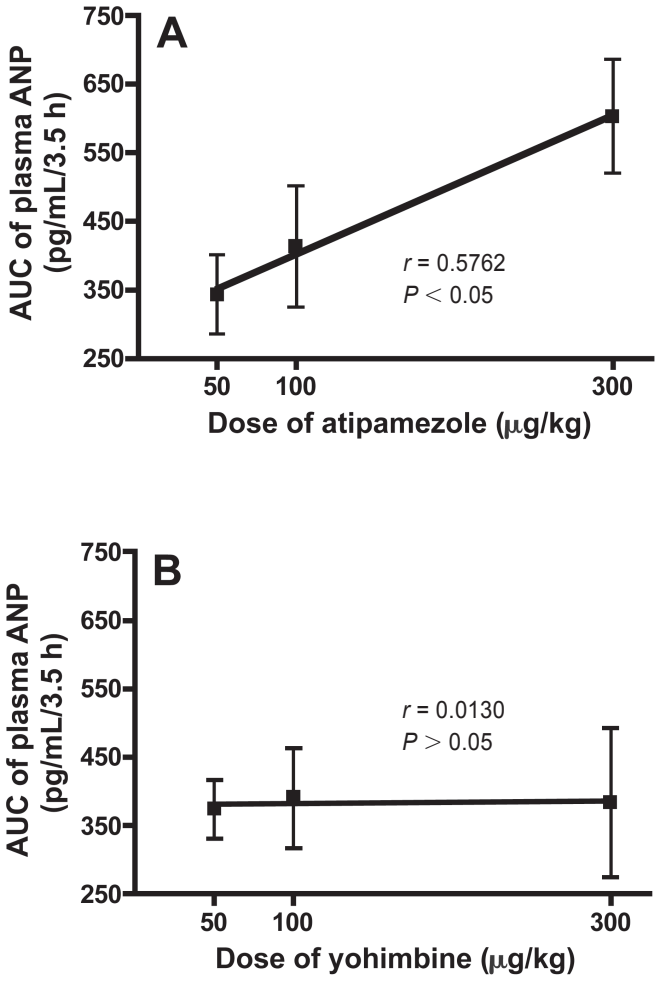

Figure 6. Simple linear regression of the AUC data for the plasma concentration of atrial natriuretic peptide (ANP) between 0.5 and 4 h after the injection of saline or medetomidine.

The plasma sodium, potassium, and chloride concentrations increased significantly in the MED group compared with baseline (Table II). Both atipamezole and yohimbine prevented the increase in the plasma concentrations of sodium, potassium, and chloride induced by medetomidine.

Discussion

To the best of our knowledge, this is the first report outlining the potent antagonistic effects of both atipamezole and yohimbine on medetomidine-induced diuresis in healthy dogs and showing that the inhibition is dose-dependent with atipamezole but not with yohimbine at the tested doses. However, at the lowest dose tested, 50 μg/kg, yohimbine was more potent than atipamezole in inhibiting the diuresis. Although the inhibitory effects of yohimbine on diuresis induced by an α_2-adrenoceptor agonist have been reported in rats (21,22,28,29), there has been no report on the antidiuretic action of atipamezole. One would expect atipamezole to exert a more specific and potent antidiuretic action on medetomidine-induced diuresis than yohimbine since the α_2-adrenoceptor selectivity and specificity of atipamezole are much greater than those of yohimbine and since atipamezole also has imidazoline-receptor affinity (1–5,10,11).

The precise mechanism of the antidiuretic action of these 2 agents is unknown. It would have been helpful to have 2 more control groups, in which only atipamezole or yohimbine was injected, to determine whether these drugs inhibit the actions of medetomidine or have contrary actions of their own. However, the main purpose of this study was to examine the antagonistic effects of atipamezole and yohimbine against medetomidine; therefore, we did not create such additional groups. We believe that atipamezole strongly antagonized the medetomidine-induced diuresis compared with yohimbine because medetomidine also has imidazoline-receptor affinity (1,3,4). On the other hand, yohimbine affects serotonergic, cholinergic, dopaminergic, and GABA receptor mechanisms (10,11), and these effects could also account in part for the differences between atipamezole and yohimbine in antidiuretic action. However, it is difficult to explain the differences from the results of this study.

In this study the decreases in urine specific gravity, urine osmolality, and urine creatinine concentration were almost simultaneous with the increase in urine volume in the MED-ATI and MED-YOH groups. Higher doses of atipamezole and yohimbine hastened the recovery from the decreases in urine specific gravity, urine osmolality, and urine creatinine concentration, indicating that higher doses of both agents strongly inhibit the diuresis induced by medetomidine.

The changes in urine and plasma electrolyte concentrations also corresponded to the antidiuretic action of both atipamezole and yohimbine. Higher doses of both agents strongly prevented the increase in plasma electrolyte concentrations induced by medetomidine.

Both atipamezole and yohimbine reversed the medetomidine-induced inhibition of plasma AVP release and urine AVP excretion. Further, for atipamezole the reversal was dose-dependent, as was reversal of the diuresis, and the reversals were correlated. We chose the period 0.5 to 4 h after the injection of medetomidine for calculation of the total urine AVP excretion and for linear regression analysis since the diuretic effects persisted for up to 4 h. We chose the period 0.5 to 4 h after the injection of medetomidine for AUC calculation and linear regression analysis of the plasma AVP data because peak diuresis occurred 2 h after medetomidine administration. Elevation of the plasma AVP concentration increases water permeability in the collecting duct (30). In rats, AVP increases cellular levels of cAMP, the 2nd messenger that leads to an increase of water permeability in the inner medullary collecting duct (30), and α_2-adrenoceptor agonists inhibit vasopressin-stimulated cAMP formation and thus inhibit water permeability (31). In our study, it could be that atipamezole and yohimbine elevated the plasma AVP concentration by their reversal action, causing an increase in water absorption from the kidney and reduced urine production, and thus inhibiting medetomidine-induced diuresis. The effects of AVP on water permeability in the kidney have been found to be mediated through regulation of aquaporin-2 (AQP-2) water channels (32). In addition, cAMP has been found to be a 2nd messenger in AQP-2 gene transcription and translocation into the luminal membrane after stimulation of the V2 receptor by vasopressin (33). The α_2-adrenoceptor agonist clonidine causes a reduction of AQP-2 in

Table I. Urine sodium, potassium, and chloride concentrations in 5 healthy dogs after an intramuscular (IM) injection of saline (2.0 mL) or medetomidine (MED), 20 μg/kg, followed 0.5 h later by an IM injection of saline (MED group), 0.5 mL, atipamezole (ATI), 50, 100, or 300 μg/kg, or yohimbine (YOH), 50, 100, or 300 μg/kg

Biochemical variable	Time after initial injection (h); mean concentration ± standard error (mmol/L) (n = 5)										
and treatment group	0	0.5	1	2	3	4	5	6	7	8	24
Sodium											
Saline	272 ± 64	296 ± 116	334 ± 111	350 ± 157	396 ± 151	405 ± 159	413 ± 184	420 ± 158	417 ± 150	403 ± 128	233 ± 69
MED	245 ± 43	200 ± 38	101 ± 13	76 ± 24	143 ± 54	285 ± 68	500 ± 133	545 ± 235	557 ± 136	584 ± 158	227 ± 53
MED-ATI 50	213 ± 43	143 ± 41	100 ± 23	74 ± 14	157 ± 39	276 ± 63	479 ± 111	394 ± 80	457 ± 95	448 ± 101	264 ± 96
MED-ATI 100	246 ± 64	188 ± 47	186 ± 54	167 ± 63	207 ± 56	287 ± 48	335 ± 92	423 ± 102	392 ± 107	301 ± 57	215 ± 49
MED-ATI 300	260 ± 61	226 ± 55	206 ± 49	143 ± 25	281 ± 48	310 ± 68	328 ± 80	363 ± 90	371 ± 88	358 ± 61	234 ± 51
MED-YOH 50	205 ± 56	201 ± 45	126 ± 35	108 ± 37	238 ± 67	330 ± 104	367 ± 93	421 ± 132	429 ± 112	427 ± 138	239 ± 47
MED-YOH 100	243 ± 76	209 ± 53	187 ± 60	109 ± 22	387 ± 83	338 ± 81	511 ± 98	479 ± 83	493 ± 116	563 ± 129	235 ± 53
MED-YOH 300	218 ± 54	208 ± 69	189 ± 59	128 ± 39	274 ± 92	279 ± 82	307 ± 104	311 ± 103	317 ± 89	326 ± 74	216 ± 50
Potassium											
Saline	190 ± 54	145 ± 39	139 ± 39	127 ± 32	124 ± 32	165 ± 37	179 ± 38	239 ± 57	2390 ± 51	213 ± 59	221 ± 80
MED	203 ± 43	132 ± 35	81 ± 43	23 ± 7[a]	36 ± 13[a]	102 ± 29	292 ± 64	340 ± 68	386 ± 112	379 ± 75	254 ± 58
MED-ATI 50	147 ± 37	120 ± 30	35 ± 8[b]	36 ± 11[b]	52 ± 13[a]	162 ± 58	388 ± 114	336 ± 99	437 ± 122[a]	408 ± 114	292 ± 77
MED-ATI 100	257 ± 76	181 ± 83	140 ± 76	143 ± 70	147 ± 57[a]	151 ± 31	242 ± 76	379 ± 93	353 ± 90	318 ± 62	278 ± 88
MED-ATI 300	234 ± 65	194 ± 60	87 ± 22	143 ± 31	222 ± 51	183 ± 48	290 ± 97	226 ± 61	304 ± 83	335 ± 79	274 ± 43
MED-YOH 50	156 ± 42	131 ± 40	64 ± 27	42 ± 13	76 ± 23	151 ± 43	158 ± 39	179 ± 50	168 ± 53	175 ± 42	224 ± 42
MED-YOH 100	191 ± 53	170 ± 37	69 ± 20	93 ± 28	205 ± 49	200 ± 50	299 ± 79	248 ± 54	220 ± 54	208 ± 31	219 ± 45
MED-YOH 300	181 ± 43	150 ± 37	104 ± 22	115 ± 31	167 ± 31	140 ± 33	103 ± 18	92 ± 15	118 ± 31	118 ± 27	218 ± 48
Chloride											
Saline	276 ± 67	230 ± 62	312 ± 114	336 ± 112	342 ± 123	342 ± 85	361 ± 137	364 ± 134	379 ± 155	402 ± 140	242 ± 74
MED	262 ± 69	226 ± 58	94 ± 15	41 ± 11[a]	94 ± 38	195 ± 54	544 ± 156	549 ± 153	636 ± 198[a]	502 ± 158	235 ± 86
MED-ATI 50	260 ± 96	182 ± 72	58 ± 19	41 ± 14[a]	110 ± 35	220 ± 59	506 ± 153	389 ± 120	416 ± 113	393 ± 115	247 ± 63
MED-ATI 100	281 ± 73	215 ± 79	173 ± 84	186 ± 64	186 ± 58	219 ± 63	379 ± 133	499 ± 160	433 ± 159	360 ± 98	236 ± 62
MED-ATI 300	273 ± 59	243 ± 49	148 ± 18	154 ± 46	241 ± 67	285 ± 64	337 ± 52	396 ± 113	353 ± 86	353 ± 73	240 ± 36
MED-YOH 50	233 ± 54	156 ± 29	62 ± 19[b]	70 ± 25[a]	196 ± 46	205 ± 54	232 ± 65	256 ± 41	310 ± 41	340 ± 78	270 ± 69
MED-YOH 100	280 ± 96	233 ± 53	103 ± 24	162 ± 56	358 ± 109	339 ± 95	434 ± 100	374 ± 67	362 ± 87	398 ± 67	246 ± 53
MED-YOH 300	241 ± 46	154 ± 32	70 ± 24	177 ± 86	233 ± 81	227 ± 59	230 ± 92	244 ± 98	256 ± 67	265 ± 85	253 ± 59

[a] Significantly different from the baseline value (0 h) at $P < 0.05$.

[b] Significantly different from the baseline value (0 h) at $P < 0.01$.

Table II. Plasma sodium, potassium, and chloride concentrations in the same experiment

Biochemical variable and treatment group	Time after initial injection (h); mean concentration ± standard error (mmol/L) (n = 5)										
	0	0.5	1	2	3	4	5	6	7	8	24
Sodium											
Saline	151 ± 27	151 ± 27	151 ± 27	151 ± 27	151 ± 27	151 ± 27	151 ± 27	151 ± 27	151 ± 27	151 ± 27	151 ± 27
MED	152 ± 3	152 ± 4	154 ± 4	157 ± 3	160 ± 4[a]	160 ± 3[a]	158 ± 4	158 ± 3	155 ± 3	154 ± 3	152 ± 2
MED-ATI 50	152 ± 2	152 ± 1	156 ± 5	156 ± 2	155 ± 4	159 ± 1[a]	156 ± 2	155 ± 1	155 ± 3	157 ± 5	152 ± 1
MED-ATI 100	152 ± 27	152 ± 27	152 ± 27	153 ± 27	154 ± 27	154 ± 27	153 ± 27	153 ± 28	153 ± 28	152 ± 28	151 ± 28
MED-ATI 300	151 ± 27	151 ± 27	153 ± 28	152 ± 27	152 ± 27	153 ± 27	153 ± 28	153 ± 28	154 ± 28	152 ± 27	151 ± 27
MED-YOH 50	152 ± 2	152 ± 2	155 ± 5	157 ± 1	156 ± 2	156 ± 2	155 ± 1	156 ± 3	155 ± 3	155 ± 4	152 ± 1
MED-YOH 100	151 ± 27	151 ± 27	152 ± 27	153 ± 27	154 ± 27	154 ± 27	153 ± 27	152 ± 27	153 ± 27	152 ± 27	151 ± 28
MED-YOH 300	151 ± 27	152 ± 27	153 ± 28	153 ± 27	152 ± 27	153 ± 27	152 ± 27	152 ± 27	152 ± 28	151 ± 27	152 ± 27
Potassium											
Saline	4.3 ± 0.8	4.3 ± 0.7	4.4 ± 0.8	4.3 ± 0.8	4.2 ± 0.7	4.3 ± 0.8	4.1 ± 0.7	4.0 ± 0.7	4.0 ± 0.7	4.0 ± 0.7	4.1 ± 0.7
MED	4.6 ± 0.2	4.8 ± 0.1	5.1 ± 0.2	5.7 ± 0.3[a]	5.4 ± 0.1[a]	5.2 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.4 ± 0.1
MED-ATI 50	4.4 ± 0.8	4.5 ± 0.8	5.0 ± 0.9	5.0 ± 0.9	4.9 ± 0.9	4.6 ± 0.8	4.5 ± 0.8	4.3 ± 0.8	4.2 ± 0.8	4.2 ± 0.8	4.3 ± 0.8
MED-ATI 100	4.5 ± 0.8	4.4 ± 0.8	4.9 ± 0.9	4.8 ± 0.9	4.7 ± 0.8	4.6 ± 0.8	4.4 ± 0.8	4.4 ± 0.8	4.2 ± 0.8	4.4 ± 0.8	4.3 ± 0.8
MED-ATI 300	4.5 ± 0.8	4.5 ± 0.8	4.6 ± 0.8	4.6 ± 0.8	4.5 ± 0.8	4.6 ± 0.8	4.5 ± 0.8	4.5 ± 0.8	4.6 ± 0.8	4.6 ± 0.8	4.4 ± 0.8
MED-YOH 50	4.5 ± 0.8	4.5 ± 0.8	4.7 ± 0.8	4.7 ± 0.8	4.7 ± 0.8	4.4 ± 0.8	4.1 ± 0.7	4.1 ± 0.7	4.0 ± 0.7	4.0 ± 0.7	4.1 ± 0.7
MED-YOH 100	4.4 ± 0.8	4.5 ± 0.8	4.6 ± 0.8	4.6 ± 0.8	4.6 ± 0.8	4.5 ± 0.8	4.3 ± 0.8	4.3 ± 0.8	4.1 ± 0.7	4.1 ± 0.7	4.4 ± 0.8
MED-YOH 300	4.5 ± 0.8	4.5 ± 0.8	4.4 ± 0.8	4.6 ± 0.8	4.5 ± 0.8	4.4 ± 0.8	4.3 ± 0.8	4.3 ± 0.8	4.4 ± 0.8	4.3 ± 0.8	4.3 ± 0.8
Chloride											
Saline	116 ± 21	114 ± 20	114 ± 20	114 ± 21	115 ± 20	115 ± 21	117 ± 21	116 ± 21	118 ± 21	117 ± 21	119 ± 21
MED	118 ± 4	119 ± 4	126 ± 1	128 ± 5[a]	127 ± 2	123 ± 2	122 ± 22	121 ± 3	121 ± 2	119 ± 3	121 ± 2
MED-ATI 50	118 ± 21	117 ± 21	117 ± 21	118 ± 21	118 ± 21	118 ± 21	122 ± 21	117 ± 22	121 ± 22	120 ± 22	119 ± 21
MED-ATI 100	117 ± 21	117 ± 21	118 ± 21	119 ± 22	119 ± 21	118 ± 21	119 ± 21	118 ± 21	119 ± 21	119 ± 22	118 ± 21
MED-ATI 300	117 ± 21	117 ± 21	117 ± 21	120 ± 21	119 ± 21	119 ± 21	118 ± 21	118 ± 21	117 ± 21	117 ± 21	117 ± 21
MED-YOH 50	117 ± 1	118 ± 1	120 ± 2	121 ± 2	122 ± 2	120 ± 1	120 ± 1	119 ± 2	119 ± 2	119 ± 1	120 ± 2
MED-YOH 100	116 ± 2	116 ± 2	115 ± 2	116 ± 2	117 ± 2	116 ± 2	116 ± 2	117 ± 2	117 ± 1	118 ± 2	118 ± 1
MED-YOH 300	116 ± 21	116 ± 21	116 ± 21	116 ± 21	119 ± 23	117 ± 21	118 ± 21	118 ± 21	118 ± 21	118 ± 21	120 ± 22

[a] Significantly different from the baseline value (0 h) at $P < 0.05$.

whole kidney as early as 1 h after administration (34). In our study, atipamezole and yohimbine may have exerted action on the AQP-2 water channels to antagonize the medetomidine-induced diuresis. On the other hand, they may have antagonized the medetomidine inhibition of plasma AVP through actions mediated via the central nervous system (2,5,7). Therefore, the reversal effects of both atipamezole and yohimbine on medetomidine's inhibition of plasma AVP release may be in part influenced by the antidiuretic actions of these agents. Our results further suggest that AVP plays a role in the diuretic effect of medetomidine (16). Moreover, we do not rule out the possibility of the involvement of peripheral factors by which these antagonists exert their antidiuretic action, as suggested by earlier reports (28,29). However, it is difficult to explain the mechanism of the antidiuretic action from the results of the present study.

This study has revealed for the first time that atipamezole treatment increases plasma ANP release significantly and dose-dependently, in contrast to yohimbine. Atipamezole potently antagonized, in a dose-dependent manner, the medetomidine-induced diuresis in the presence of an increased plasma ANP concentration. On the other hand, all the tested doses of yohimbine strongly antagonized the medetomidine-induced diuresis without an elevated plasma ANP concentration. Therefore, it may be that ANP has a weak diuretic action in medetomidine-induced diuresis and that atipamezole has an agonistic effect on ANP release. The level of ANP stimulated by atipamezole at the tested doses may not have been enough to induce diuresis. The stimulation of plasma ANP release has been reported to relate to the atrial stretch due to hypertension (20–25). Atipamezole produces stronger and relatively longer-lasting hypertension (7,9–11). This may be a cause of the ANP release stimulated by atipamezole in this study.

In conclusion, both atipamezole and yohimbine had profound antagonistic effects on medetomidine-induced diuresis in healthy dogs. Although yohimbine did not inhibit the diuresis in a dose-dependent manner, in contrast to atipamezole, it had potent inhibitory action at all tested doses. The results suggest that AVP plays a role in the antidiuretic effects of both atipamezole and yohimbine. This study also demonstrates for the first time that atipamezole stimulates ANP release significantly. The differences in the antidiuretic mechanism of atipamezole and yohimbine may be due to the differences in their selectivity and specificity for the α_2-adrenoceptor subtypes and imidazoline receptors. In addition, other cellular messengers, other receptor subtypes, and the AQP-2 water channels may have biologic roles in the antidiuretic action of atipamezole and yohimbine, since urine is the net product of multiple hemodynamic, neural, hormonal, and local factors in the kidney. However, the precise mechanism cannot be explained from the results of this study. Both drugs can be used to antagonize medetomidine-induced diuresis in healthy dogs.

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